THE SIGNIFICANCE OF CERTAIN WOOD-DESTROYING FUNGI IN THE STUDY OF THE ENZYMATIC HYDROLYSIS OF CELLULOSE

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With plates 209 and 210 and three text figures

INTRODUCTION

IN 1913, one of us[1] called attention to certain wood-destroying fungi which produce helically oriented cavities within the thick secondary walls of the latewood of *Pinus Taeda* L. Subsequently, in studying the comparative anatomy of a wide range of conifers, monocotyledons, and dicotyledons, we have encountered similar fungi, not only in the wood of many different species, genera and families of the higher plants, but also in material from diverse temperate and tropical environments. The fungi evidently are ubiquitous forms which attack the woody tissues of the gymnosperms and of the angiosperms when these tissues are cut and are exposed to the air.

The fungi are characterized by the facts (1) that at least a part of their hyphae move forward within the secondary wall and (2) that their enzymes dissolve cavities which are oriented either helically or parallel to the long axis of the cell. The arrangement of the enzymatically-produced cavities suggests that hydrolysis proceeds along planes that are determined by the structural orientation of the cellulose, and, therefore, that such fungi may afford a means of securing significant information regarding predetermined planes of chemical reaction in the cellulosic matrix of the secondary wall. The results of a reconnaissance of woods that have been attacked by these fungi[2] are recorded in the following pages.

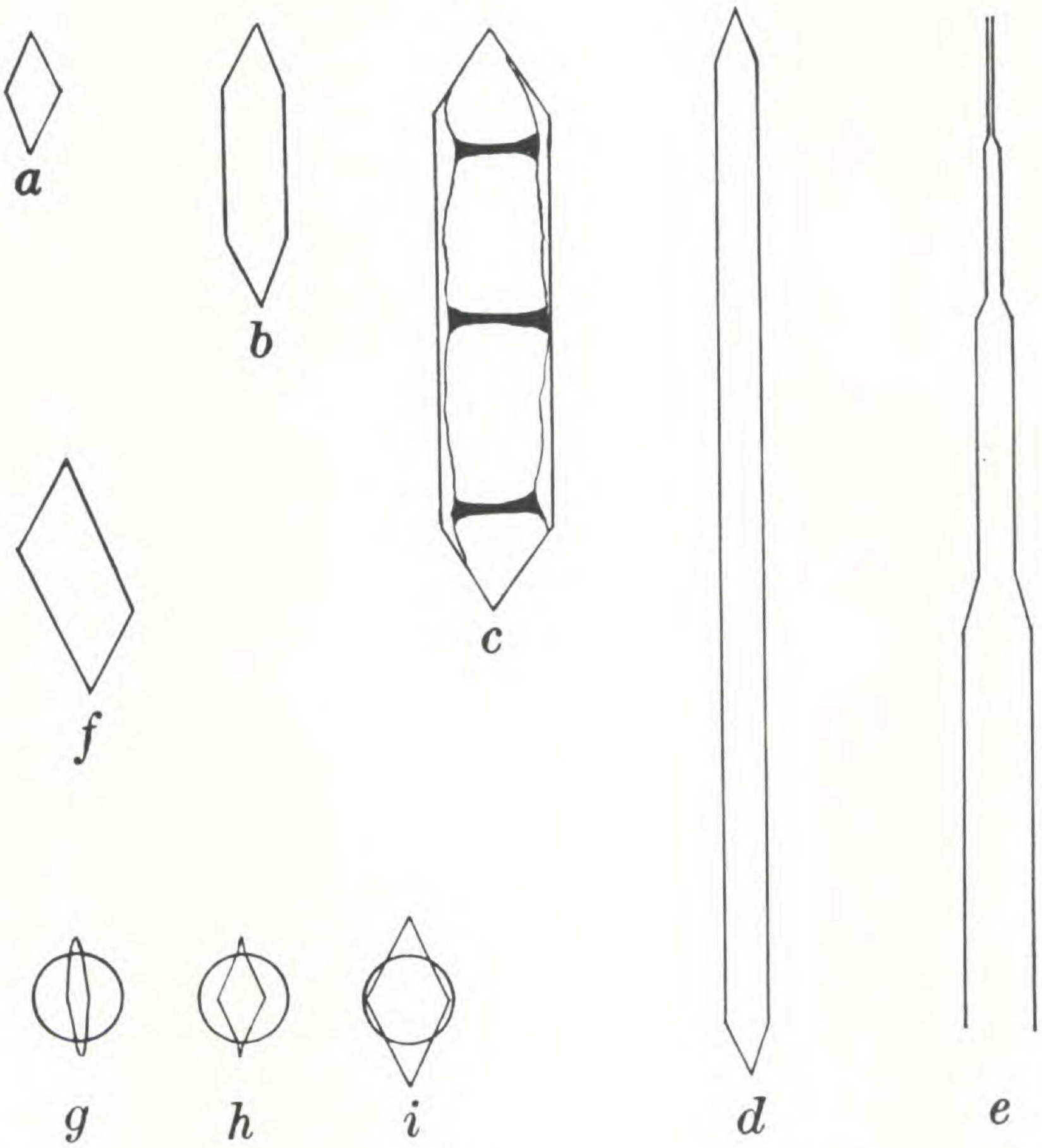
FORM AND DEVELOPMENT OF THE ENZYMATICALLY-PRODUCED CAVITIES

During their stages of elongation, the hyphae are extremely tenuous filaments which dissolve correspondingly minute, elongated cavities

[1]BAILEY, I. W., The preservative treatment of wood. I. The validity of certain theories concerning the penetration of gases and preservatives into wood (Forestry Quarterly **11**: 5-11. 1913).

[2]We have found these fungi in 114 species, 88 genera, and 36 families of the gymnosperms and angiosperms.

within the secondary wall (Text fig. 3). These slender, cylindrical perforations subsequently are enlarged by further enzymatic activity (Pl. 209, Figs. 1, 2, 6 and 7) which may continue until much of the secondary wall is dissolved (Pl. 209, Fig. 3). The process of lateral

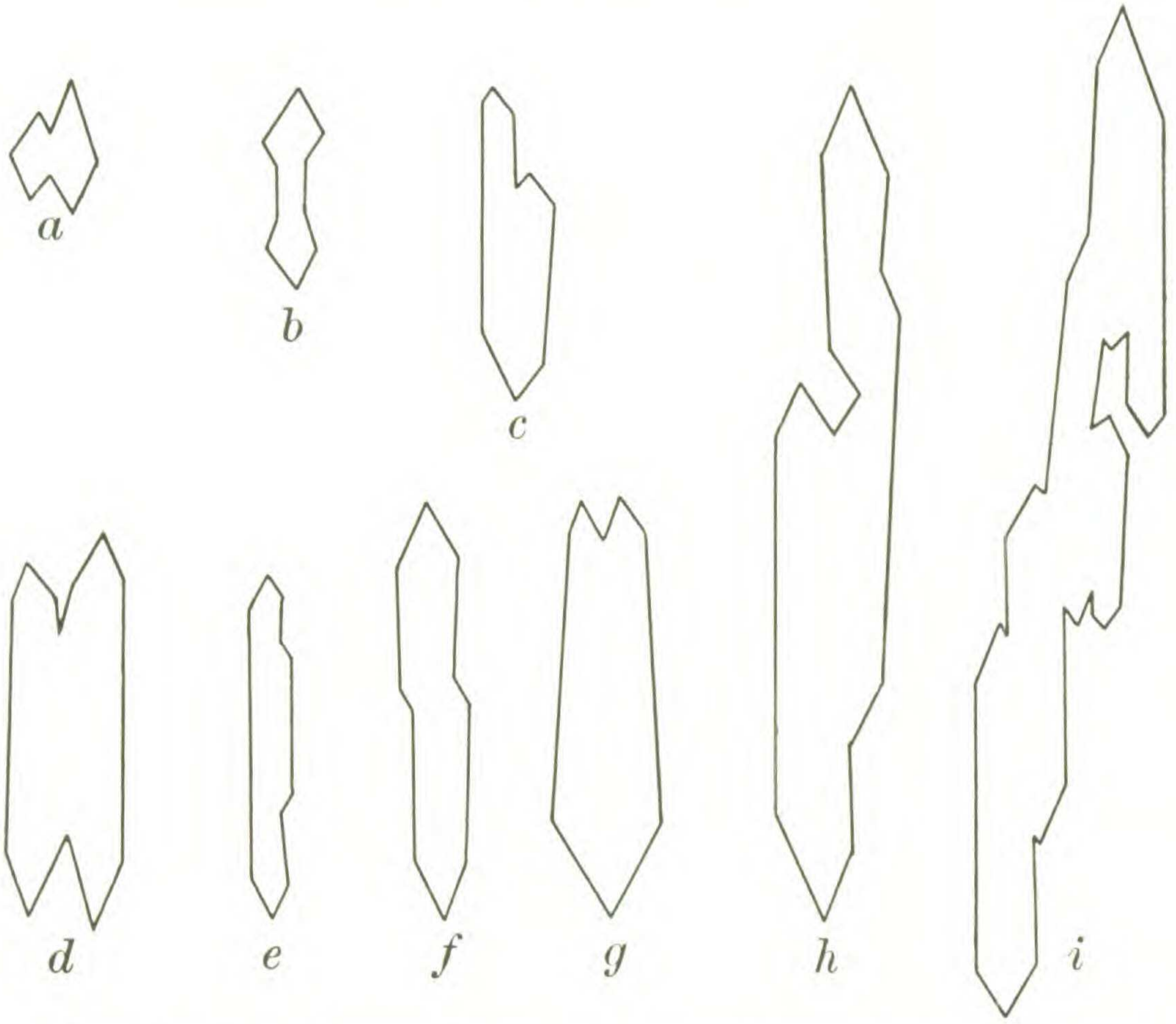


TEXT FIGURE 1. Enzymatically-produced cavities as seen in median longitudinal planes of optical section. (a) Biconical cavity. (b), (c) and (d) Cylindrical cavities with conical ends, (c) containing remains of dilated hypha. (e) Progressive stages in the enlargement of a slender cylindrical perforation. (f) Cavity produced by the lateral fusion of two biconical cavities. (g), (h) and (i) Successive stages in the enlargement of a pit orifice.

enlargement rarely progresses uniformly throughout the length of the cylindrical cavities, but tends to be accelerated in certain parts and to be retarded in others. Thus, as indicated in Pl. 210, Figs. 8–13, localized enzymatic activity produces more or less numerous dilations which are

oriented in a linear series and are connected by unaltered or less modified parts of the original elongated perforation.

Although the chambers vary considerably in size, they obviously are restricted to two principal geometric forms (1) biconical or (2) cylindrical with conical ends. In perfectly median longitudinal sections, the former cavities have a diamond-shaped outline (Pl. 210, Figs.

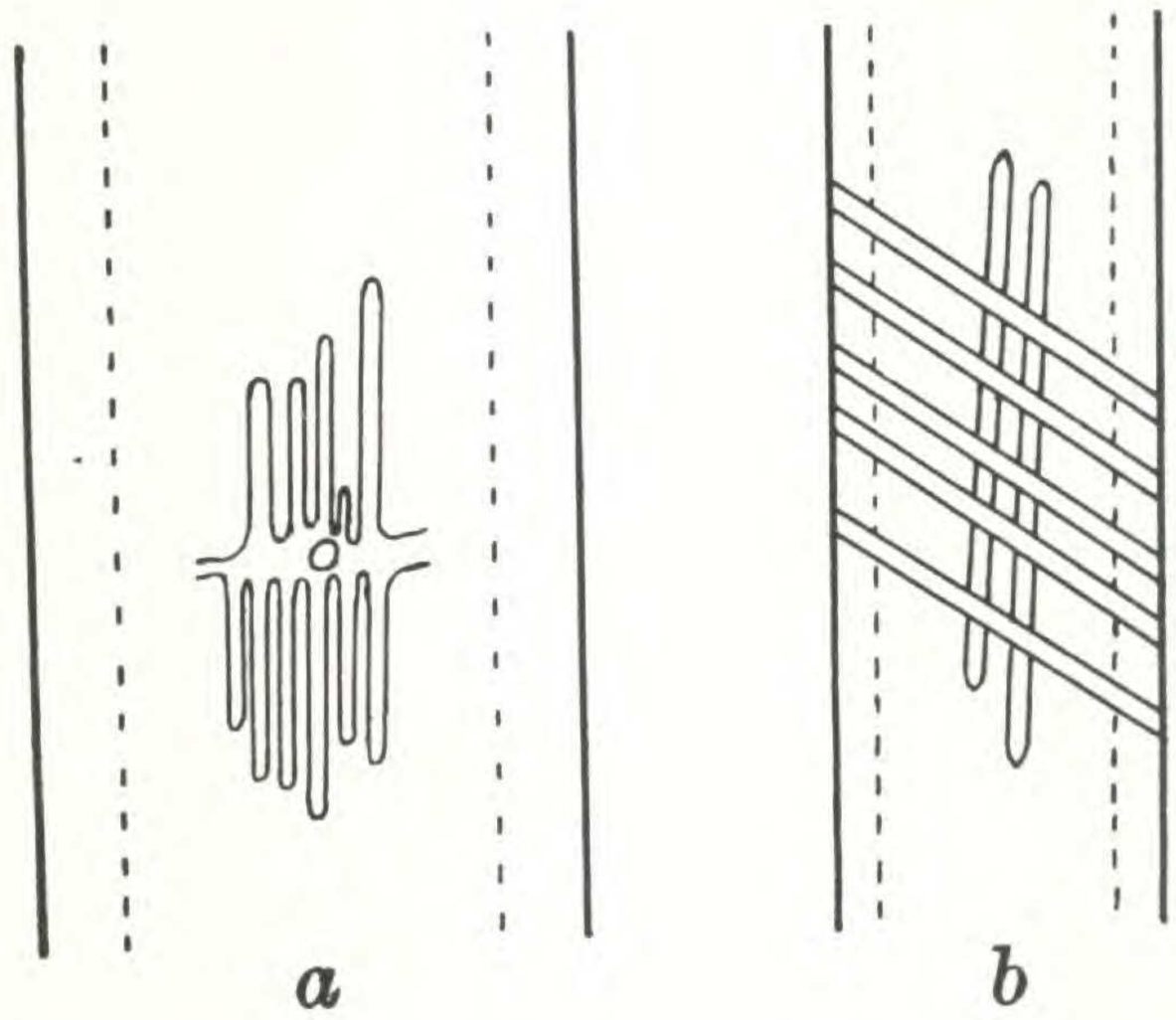


TEXT FIGURE 2. Complex types of cavities resulting from the fusion of primary forms.

8, 10 and 11), whereas the latter have parallel sides and terminate in acute angles (Pl. 210, Figs. 9 and 13; Text fig. 1, b, c and d). Of course, the outlines deviate considerably in other planes of section (Pl. 210, Fig. 12) and many complex forms arise during the fusion of cavities, either of the same linear series (Text figs. 1, e and 2, b) or of adjacent series (Text figs. 1, f and 2, a, c-i).

Such facts as these suggest that hydrolysis of the cellulose through enzymatic activity proceeds along two clearly defined sets of planes. During elongation of the tenuous hyphae, *terminal* enzymatic activity

progresses primarily along one of the sets of planes producing attenuated, cylindrical perforations, whereas subsequent *lateral* enzymatic activity develops along either or both of these sets of planes and produces biconical cavities or cylindrical chambers with conspicuously conical ends.



TEXT FIGURE 3. (a) Early stage of the growth of hyphae within the central layer of the secondary wall. (b) Helically oriented perforations in the outer layer of the secondary wall in contrast to the more nearly vertical arrangement in the central layer.

ORIENTATION OF THE PLANES OF ENZYMATIC HYDROLYSIS

As a result of varied optical and physico-chemical investigations, it is now generally recognized that, in the case of tracheids and fibers, the chain molecules of cellulose are oriented approximately parallel to the long axis of the anastomosing fibrils which constitute the secondary wall. That the long axis of the hyphal filaments and of the cylindrical perforations is oriented parallel to that of the fibrils and, therefore, to that of the chain molecules or micelles, may be demonstrated by various lines of evidence. (1) Where the fibrillar structure of the secondary wall is clearly visible, as is sometimes the case, it may be observed that the hyphae and the linear series of cavities are oriented parallel to the long axis of the fibrils. (2) In thick-walled tracheids, fiber-tracheids, and libriform fibers of the normal 3-layered type, the slitlike orifices of the pits are oriented parallel to the fibrils of the broad central layer of the secondary wall. Since most of the enzymatically-produced cavities are confined to this layer (Pl. 209, Figs. 1 and 2) the pit orifices afford a

reliable means of correlating the orientations of the fibrils and of the cylindrical perforations within the central layer of the secondary wall. (3) In thin, 5 μ, longitudinal sections of favorable material, it may be demonstrated that the positions of extinction of the cellulose in polarized light are oriented parallel to the sides of the cylindrical cavities. (4) When sections of lignified secondary walls are chlorinated, treated with an aqueous solution of iodine-potassium iodide, and subsequently with a drop of 60% sulphuric acid, dark brown crystals of iodine form within the elongated interstices of the cellulosic matrix. These elongated crystals, or crystalline aggregates, are visible microscopically and are oriented parallel to the long axis of the fibrils. By means of these crystals, it is possible not only to detect such major variations in the structural orientation of cellulose as occur in passing from layer to layer of the secondary wall, but also to observe such minor fluctuations in orientation as occur within the limits of a single layer. As shown in Pl. 209, Fig. 5, the linear series of cavities and the sides of the individual cylindrical chambers are oriented parallel to the long axis of the crystals, and therefore of the fibrils and chain molecules.

These and other lines of corroborative evidence indicate that the hyphae, the cylindrical perforations, and the linear series of enzymatically-produced cavities are oriented parallel to the long axis of the cellulosic fibrils. Where the chain molecules, the micelles, and the fibrils are helically oriented, the hyphae and the cavities have a helical arrangement (Pl. 210, Figs. 12 and 13) and where they are oriented more nearly parallel to the long axis of the cell, the hyphae and the perforations have a similar arrangement (Pl. 210, Figs. 8, 9 and 10). Furthermore, where the orientation of the cellulose changes in different parts of the secondary wall, the arrangement of the hyphae and of the enzymatically-produced cavities fluctuates accordingly (Text fig. 3, b). In other words, one set of the predetermined planes of enzymatic activity is oriented parallel to the long axis of the fibrils, and, therefore, of the chain molecules of cellulose. The second set of planes obviously is oriented at an acute angle to this axis, and it is essential to measure the angle and to determine, if possible, whether it is variable or constant.

Unfortunately, there are inherent optical and other difficulties to be overcome in measuring this angle with consistent accuracy. In the first place, there is the difficulty of turning the lines of the eyepiece into exact coincidence with the two legs of the angle to be measured. The smaller the cavity, the greater this source of error becomes. In the second place, there is considerable uncertainty in determining whether a particular cavity is being viewed in a truly median longitudinal plane of optical section. This difficulty is accentuated by the fact that, in

the case of helically oriented structures, the chambers are curved, and furthermore by the fact that many of the larger cavities are not truly cylindrical or biconical, i. e., they are not perfectly circular in transverse sections (Pl. 209, Figs. 1, 2 and 6). Another common source of error, particularly in dealing with the larger cavities, is local deviations in the orientation of the cellulose, which lead to the formation of asymmetrical cavities. It is significant, in addition, that the critical angles become smaller when the wall contracts, e. g., in drying, and enlarge when the the wall swells. Thus, the angles may be modified during the processes of drying, and subsequently of resoaking, softening, and dehydrating the material for microscopic examination.

The measurements recorded in Table 1 were obtained from nine species and genera of seven different families, including one gymnosperm. Although the individual measurements for particular species, and the averages for different species, fluctuate through a range of variation of from 5 to 6 degrees, it is reasonable to assume that many of these variations are due to inherent difficulties in accurately measuring the angles of intersection of the two sets of planes. Thus, there appear to be two predetermined sets of planes of enzymatic activity in the secondary walls of tracheary cells and fibers, (1) oriented parallel to the long axis of the chain molecules and fibrils and (2) oriented at an angle of from 20–25 degrees to this axis.

TABLE 1

MEASUREMENTS OF THE ANGLE OF INTERSECTION OF THE TWO PRINCIPAL PLANES OF ENZYMATIC ACTIVITY

Plant	Min.	Av.	Max.
Ilex formosana Maxim.	15.0	19.5	21.8
Myodocarpus fraxinifolius Brongn. & Gris.	16.9	19.5	22.1
Iryanthera macrophylla (Benth.) Warb.	18.9	20.5	21.8
Laurelia aromatica Juss.	18.3	22.7	24.3
Adinandra sp.	21.0	22.8	24.9
Cussonia Barteri Seem.	19.9	23.2	26.0
Osteophloeum platyspermum (A. DC.) Warb.	20.3	23.2	25.6
Pinus echinata Mill.	18.8	23.7	27.5
Brackenridgea Hookeri A. Gray	22.9	25.6	28.1
Average		22.3	

Basis of individual averages, 20 measurements.

The second set of planes is not correlated with any visible structures of the cellulosic matrix and, therefore, is determined by submicroscopic ones. The fact that the orientation of these planes is modified during

the swelling of the secondary wall, — i. e., where the spacing of the glucose residues is altered — suggests that these planes of hydrolysis are determined by specific configurations in the unit cell of cellulose.

It is possible to isolate fibers that have been attacked by these fungi, and subsequently to treat them with reagents — e. g., sulphuric acid, phosphoric acid or cuprammonium hydroxide — which dissect the wall into "fusiform bodies"[1] and other minute anisotropic fragments. By observing the phenomena in close proximity to the enzymatically-produced cavities, it may be observed that the fusiform bodies are dissected from the wall along planes that are parallel to those of these cavities. In other words, the chemical changes induced by these inorganic reagents progress along planes that are oriented parallel to the predetermined planes of enzymatic activity. As the fusiform bodies are cut free from the wall, they tend to be more or less rapidly deformed by the swelling effects of the reagents used in their production. When the walls themselves are swollen during the treatment — i. e., where the spacing of the chain molecules is altered — the angles between the two intersecting sets of planes are increased, and the shape of the enzymatically-produced cavities becomes correspondingly modified. Under such circumstances, the original cavities contract longitudinally and expand laterally. Thus, the orientation of the oblique surfaces is gradually modified.

The illustrations of partially acetylated fibers published by Hess,[2] Kanamaru,[3] and others indicate that acetylation of cellulose tends to proceed along similar planes, i. e., planes parallel to the long axis of the chain molecules or fibrils, and planes set at an acute angle to this axis. A careful study of these planes of chemical action during hydrolysis and acetylation should yield significant data regarding the submicroscopic configurations of cellulose.

MISCELLANEOUS DATA CONCERNING THE FUNGI AND THEIR HYPHAE

In view of the fact that we have not succeeded in finding any descriptions of these fungi in the literature or information concerning their identity, it seems advisable to record the following data regarding them, even though our observations, thus far, are based solely upon the study of sections of thoroughly seasoned tissues. There are two types of hyphae in a majority of the specimens that we have examined (1) delicate, colorless filaments and (2) coarse, dark brown hyphae which con-

[1]RITTER, J. Dissection of wood fibrils by chemical means. (Ind. Eng. Chem. **21**: 289. 1929.)

[2]HESS, K. Die Chemie der Zellulose und ihrer Begleiter. Leipzig. 1928.

[3]KANAMARU, K. Die Brechungsindices von Nitrocellulose und Acetylcellulose. (Helv. Chem. Acta **17**: 1429-1440. 1934.)

nect with them. Both types of hyphae are septate, and both are devoid of obvious clamp-connections. The abundance and distribution of the hyphae vary greatly from specimen to specimen. In certain cases, the colored hyphae are confined largely to the lumina of the rays and wood parenchyma — as is true for the "blue stain" fungus — whereas in others they occur chiefly in the lumina of the vessels, fiber-tracheids or libriform fibers. The colorless hyphae perforate the secondary walls and move forward within them. As the enzymatically-produced cavities enlarge, the hyphae tend to become more or less conspicuously dilated (Text fig. 1, c) and, in dried material, frequently are encrusted both internally and externally with a granular substance which stains deeply with Haidenhaín's haematoxylin (Pl. 209, Figs. 1, 2, 4 and 6).

The hyphae which move forward within the secondary wall usually attack the walls of the tracheids, fiber-tracheids or libriform fibers, and occasionally of the vessels, but rarely, if ever, of ray parenchyma or of wood parenchyma. Furthermore, they tend to develop primarily within the central layer of the secondary wall (Pl. 209, Figs. 1, 2 and 6); although in the case of certain specimens, they may perforate the outer layer as well (Text fig. 3, b). As indicated in (Pl. 209, Fig. 3), the fungi frequently dissolve the central layer of the secondary wall, leaving the inner and the outer layers intact. Such facts as these suggest that the enzymatic activity may be retarded or inhibited in walls and layers which are very intensely lignified.

In a few of the specimens, which have abundant hyphae within the lumina of the fiber-tracheids, and few, if any, hyphae within the secondary wall, the enzymes attack the inner surface of the wall, and the hydrolysis progresses centrifugally through the central layer. Although this type of enzymatic activity produces less obviously symmetrical cavities, it tends to proceed along two clearly defined sets of planes, i. e., parallel to the long axis of the fibrils and at an angle of 20–25 degrees to this axis. The latter planes of enzymatic hydrolysis are most clearly visible in walls where the orifices of the bordered pits are undergoing enlargement (Text fig. 1, g, h, i).

It should be emphasized, in conclusion, that these fungi are so significant from experimental and physico-chemical points of view that an effort should be made to isolate them, to grow them in pure cultures, and to obtain reliable information concerning their identity.

Since completing the manuscript for this paper, we have examined one of Dr. D. H. Linder's specimens of the wood of *Acer rubrum* L. which has been attacked by a species of *Brachysporium*. The hyphae of this fungus dissolve helically oriented cavities of the same geo-

metrical forms as have been described in this paper. Dr. Linder is of the opinion, after examining our slides, that we are concerned with Pyrenomycetes or with the imperfect stages of this group.

CONCLUSION

1. There are certain fungi whose hyphae perforate and move forward within the secondary walls of tracheary cells and fibers.

2. The cavities produced by these fungi are of two geometrical forms, i. e., (1) cylindrical with conical ends or (2) biconical, and are of remarkably constant angularity, regardless of the particular group of gymnosperms or angiosperms in which they occur.

3. It is evident that the enzymatic activity of these fungi progresses along two predetermined sets of planes, (1) oriented parallel to the long axis of the fibrils and chain molecules of cellulose and (2) at an angle of from 20–25 degrees to this axis.

4. These fungi evidently are ubiquitous forms which attack the vascular and fibrous tissues of the higher plants when they are cut and are exposed to the air.

5. The fungi are so significant from experimental and physicochemical points of view that an effort should be made to isolate them, to grow them in pure cultures, and to determine their identity.

DESCRIPTION OF PLATES

PLATE 209

Fig. 1. *Poraqueiba sericea* Tul. Transverse section of the xylem, showing cavities in the central layer of the secondary wall. × 1470.

Fig. 2. *Stemonurus secundiflorus* Blume. Transverse section of the xylem, showing cavities in the secondary wall. × 580.

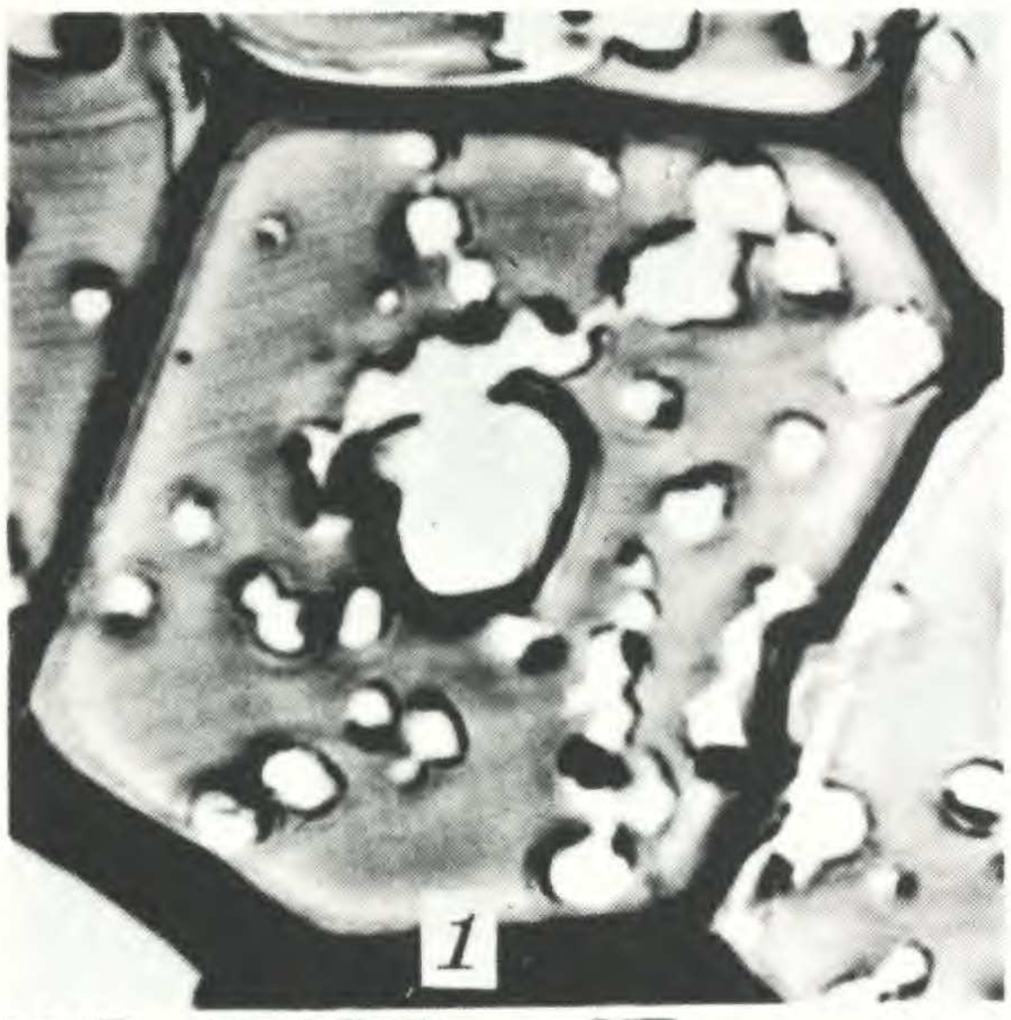
Fig. 3. *Compsoneura capitellata* (Poepp.) Warb. Transverse section of the xylem, showing an advanced stage of the decay. The central layer of the secondary wall is being removed, leaving the more heavily lignified inner and outer layers. × 760.

Fig. 4. *Iryanthera macrophylla* (Benth.) Warb. Longitudinal section of the xylem, showing enzymatically-produced cavity in the secondary wall and hypha encrusted with a deeply stainable substance. × 1470.

Fig. 5. *Brackenridgea Hookeri* A. Gray. Longitudinal section of the xylem, showing orientation of enzymatically-produced cavities and of crystalline complexes of iodine. × 760.

Fig. 6. *Laurelia aromatica* Juss. Transverse section of the xylem, showing hyphae and enzymatically-produced cavities in the secondary wall. × 1470.

Fig. 7. *Davidia involucrata* Baill. Transverse section of the xylem, showing cavities in the secondary wall. × 1470.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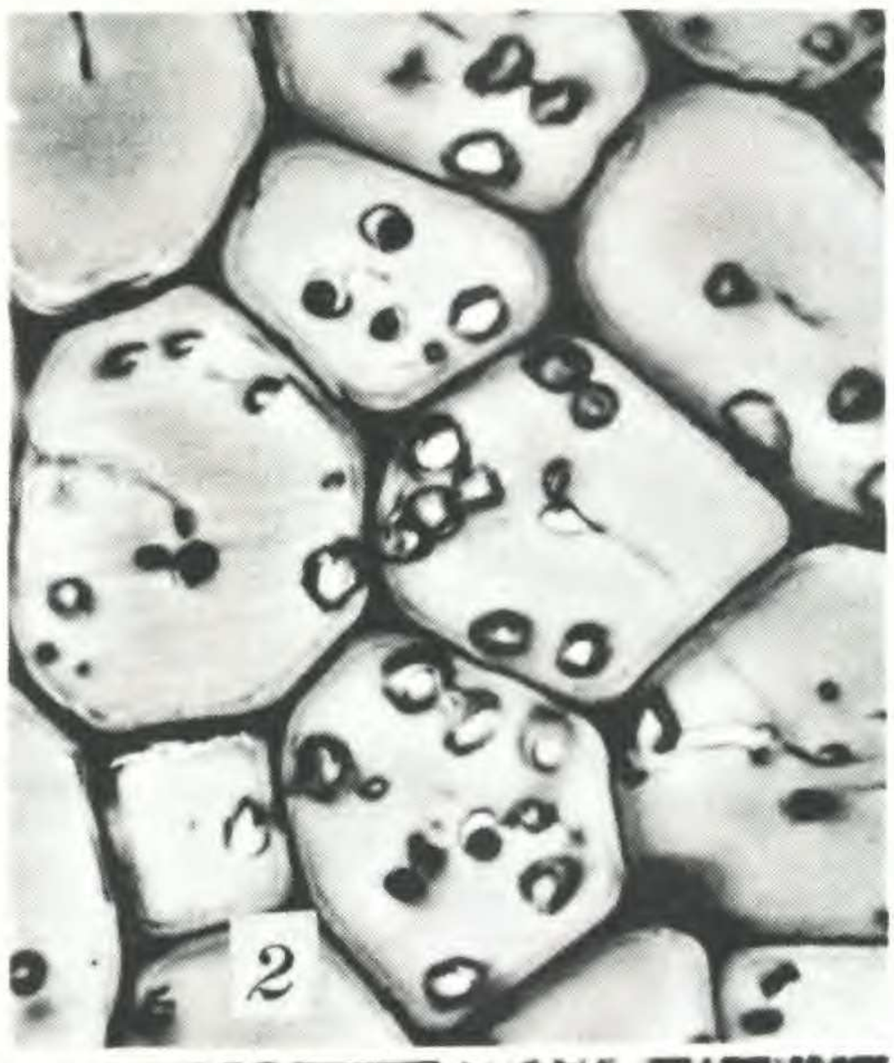
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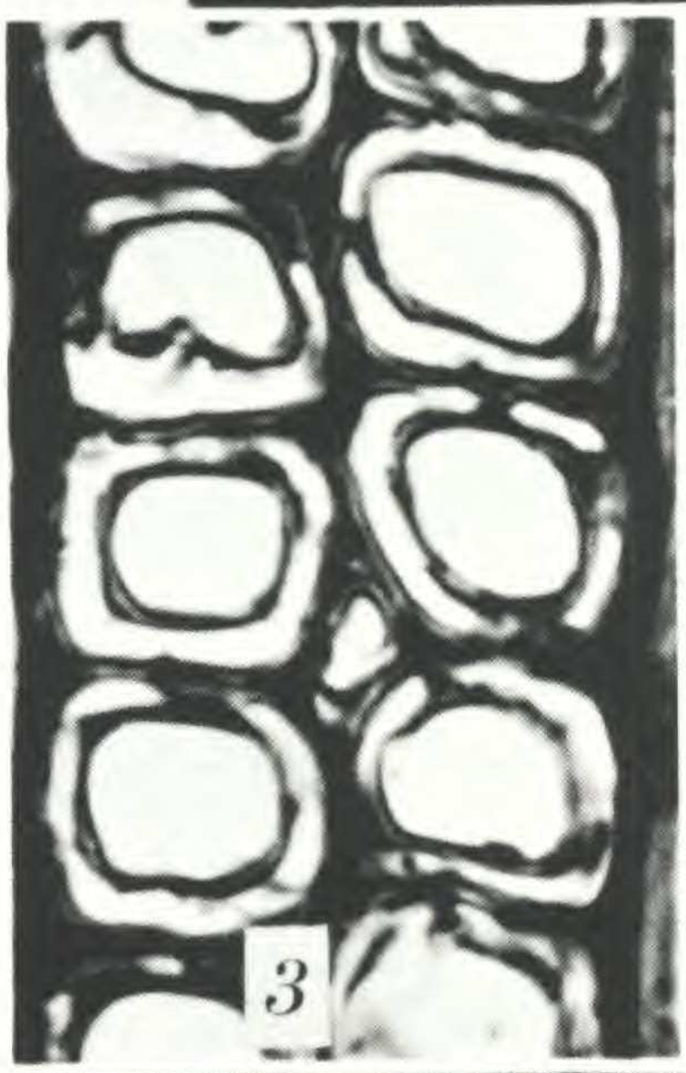
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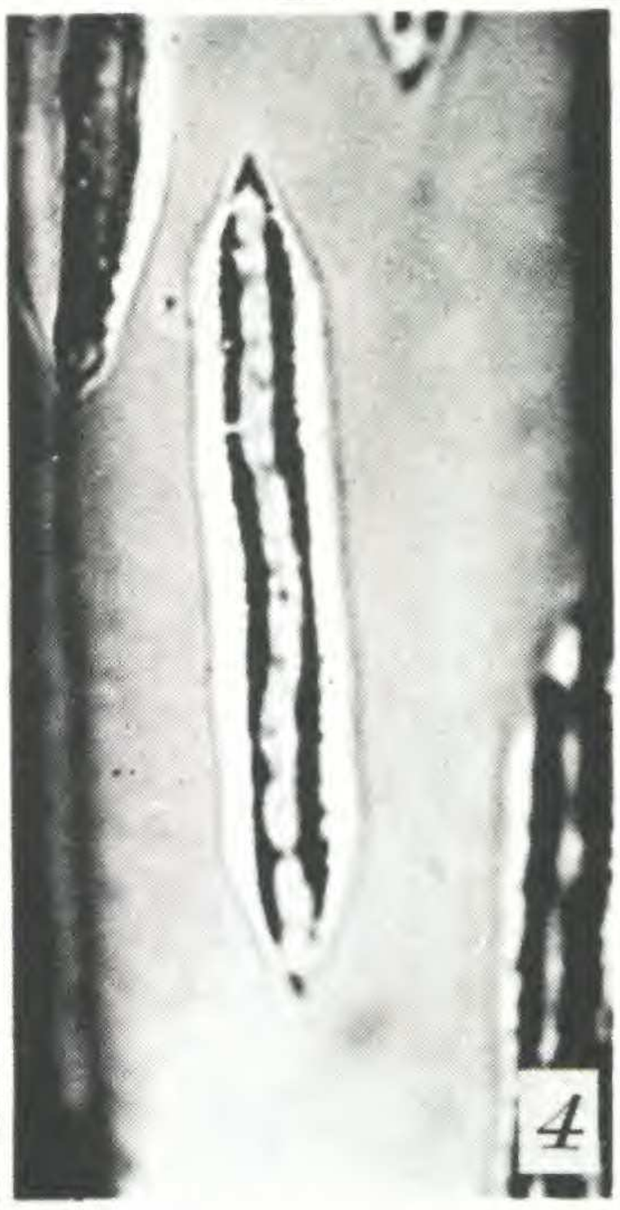
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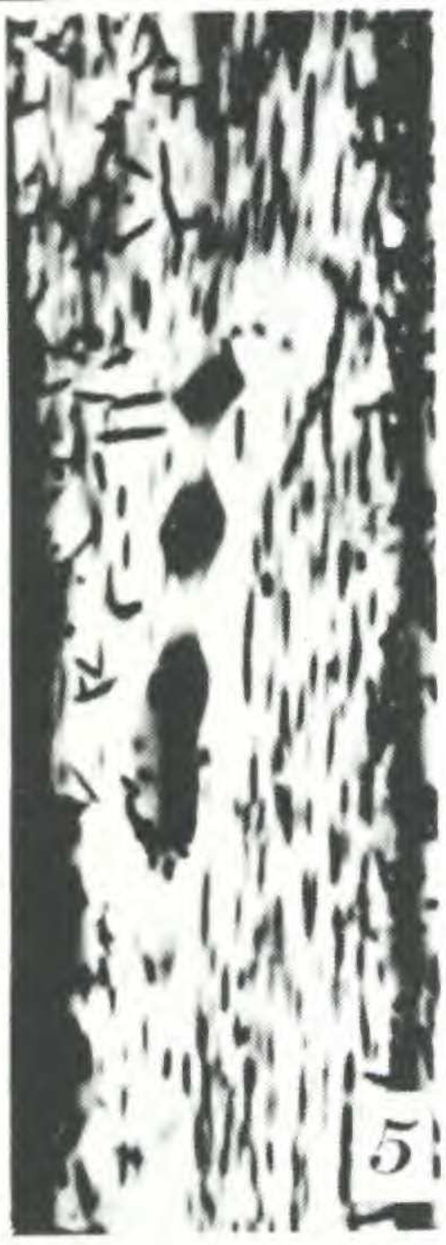
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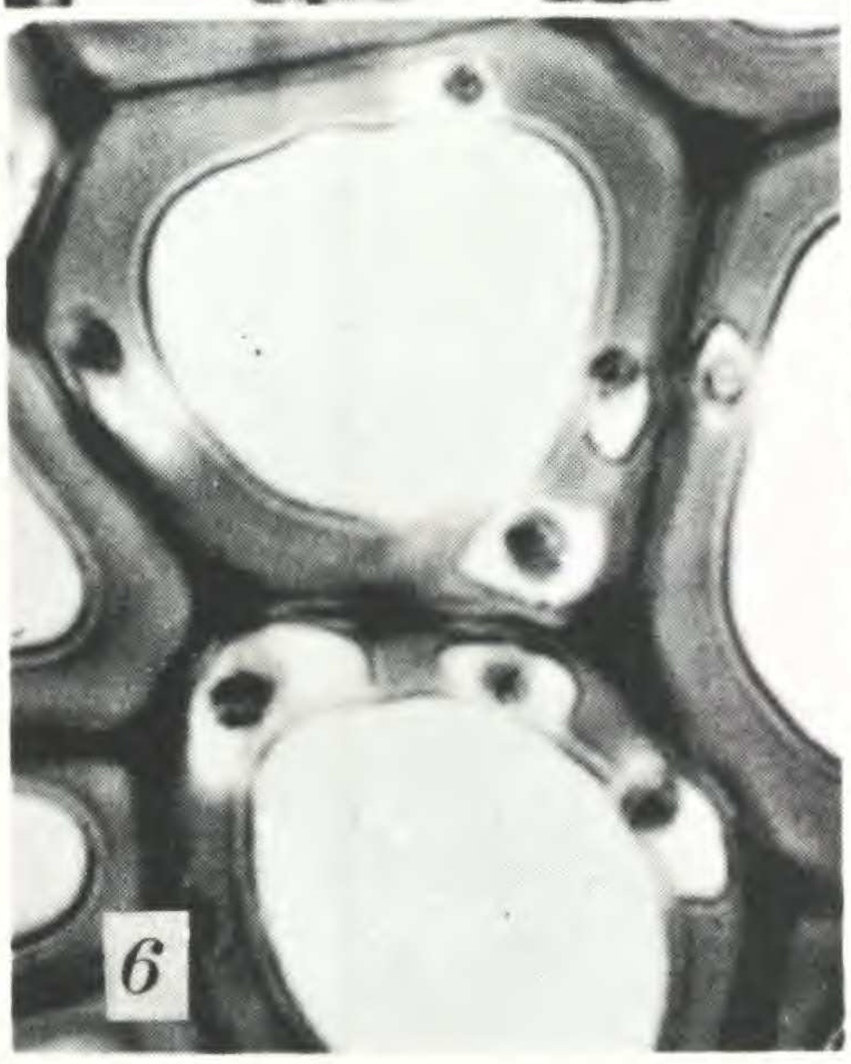
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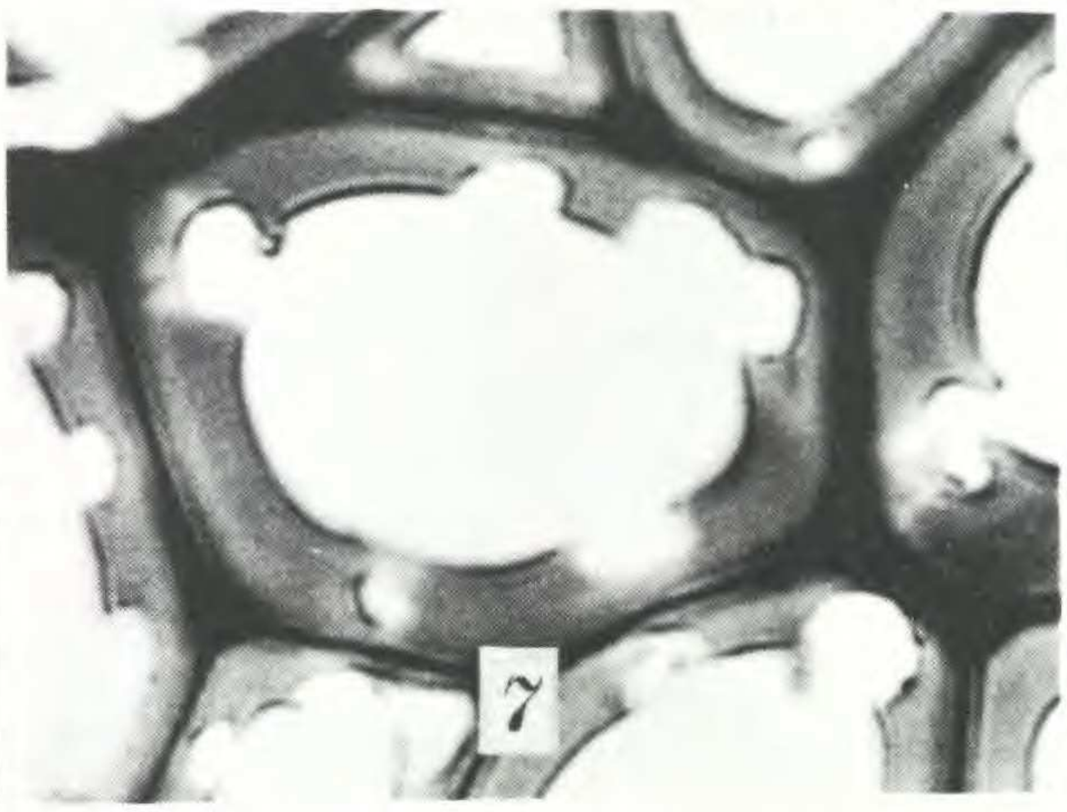
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WOOD-DESTROYING FUNGI

FULL-TONE — MERIDEN